CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

761231Orig1s000

PRODUCT QUALITY REVIEW(S)

Recommendation: Approval

BLA/NDA Number: 761231
Assessment Number: First round
Assessment Date: November 26, 2021

Drug Name/Dosage Form	ALYMSYS (bevacizumab-maly) solution for intravenous infusion
Strength/Potency	25 mg/mL
Route of Administration	Intravenous infusion
Rx/OTC dispensed	Rx
Indication	Treatment of metastatic colorectal cancer, non-squamous non-small cell lung cancer, glioblastoma, metastatic renal cell carcinoma, cervical cancer, epithelial ovarian, fallopian tube, or primary peritoneal cancer
Applicant/Sponsor	Amneal Pharmaceuticals
US agent, if applicable	N/A

Product Overview:

Quality Assessment Team:

Discipline	Assessor	Branch/Division
Drug Substance, Drug Product, Comparative Analytical Assessment, Immunogenicity	Meng-Jung Chiang	OBP/DBRR-I
Labeling	Jennifer Kim	OBP/IO
Microbiology and Facilities, DS	Charles Yuan-Chia Kuo	OPMA/DBM/BMB1
Microbiology and Facilities, DP	Wendy Tan, Chani Broner	OPMA/DBM/BMB1
Facilities QAL	Zhong Li	OPMA/DBM/BMB1
Microbiology QAL	Maxwell Van Tassell	OPMA/DBM/BMB1
Application Technical Lead	Jennifer Swisher	OBP/DBRR-IV
RBPM	Andrew Shiber	OPRO/DRBPMI/RBPMB2

Multidisciplinary Assessment Team:

Discipline	Assessor	Office/Division
RPM	Gina Davis	OND/ORO/DROOD
Cross-disciplinary Team Lead	Sandra Casak	OND/OOD/DOIII
Medical Officer	Margaret Thompson	OND/OOD/DOIII
Pharmacology/Toxicology	Dubravka Kufrin; Matthew Thompson, TL	OND/OOD/DHOT
Clinical Pharmacology	Miao Zhao; Salaheldin Hamed, TL	OTS/OCP/DCPI
Statistics	Yi Ren; Yuan-Li Shen, TL	OTS/OB/DBV

1. Names:

   a. Proprietary Name: ALYMSYS
   c. Non-Proprietary Name/USAN: bevacizumab-maly
   d. CAS Registry Number: 216974-75-3
   e. Company/Laboratory Code: MB02
   f. INN Name: bevacizumab

h. OBP systematic name: MAB HUMAN (IGG1) Anti-P15692 (VEGF-A Human) [MB02]

Submissions Assessed:

Submission(s) Assessed	Document Date
761231 Original BLA submission	4/13/2021
761231/8 (Response to IR#1)	6/4/2021
761231/16 (Response to IR#2)	7/19/2021
761231/17 (Response to IR#3)	7/28/2021
761231/30 (Response to IR#4)	10/4/2021
761231/38 (Response to IR#5)	11/12/2021
761231/39 (Response to IR#6, in response to written request from Amneal)	11/18/2021
761231/40 (Response to IR#6)	11/24/2021
761231/42 (Response to IR#7)	12/13/2021
761231/46 (Response to IR#8)	1/3/2022
761231/52 (Response to IR #6)	1/20/2022
761231/54 (Response to IR #9)	1/26/2022
761231/57 (Response to LCM comments)	3/3/2022

Quality Assessment Data Sheet:

1. Legal Basis for Submission: 351(k)

2. Related/Supporting Documents:

   A. DMFs:

DMF #	DMF Type	DMF Holder	Item referenced	Code[1]	Status[2]	Date Assessment Completed	Comments
(b) (4)	Type III		(b) (4)	3	Adequate	N/A	None
	Type III			3	Adequate	N/A	None
	Type III			3	Adequate	N/A	None

1. Action codes for DMF Table: 1- DMF Assessed; Other codes indicate why the DMF was not assessed, as follows: 2- Assessed previously and no revision since last assessment; 3- Sufficient information in application; 4- Authority to reference not granted; 5- DMF not available; 6- Other (explain under "comments")
2. Action codes for Status column: Adequate, Adequate with Information Request, Deficient, or N/A (There is not enough data in the application; therefore, the DMF did not need to be assessed.

   B. Other documents: none

3. Consults: none

4. Environmental Assessment: Amneal requests a categorical exclusion from the preparation of an environmental assessment in accordance with 21CFR25.31(a), as increased use of MB02 will not occur because the drug is not a new molecular entity and will be administered at the same dosage levels and for the same duration and indications as US-licensed Avastin. Therefore, this request for categorical exclusion is appropriate and is granted.

Executive Summary:

I. Recommendations:

A. Recommendation and Conclusion on Approvability:

Recommendation: **APPROVAL**

This BLA is recommended for approval from the perspective of microbial control, sterility assurance, and product quality by both OPMA and OBP.
The Drug Substance facility assessment recommendation is pending Pre-license Inspection (PLI) of (b) (4)

If deemed acceptable for approval, the following language can be used:

The Office of Pharmaceutical Quality, CDER, recommends approval of STN 761231 for Alympsys manufactured by Amneal Pharmaceuticals. The data submitted in this application are adequate to support the conclusion that the manufacture of Alympsys is highly similar to US-licensed Avastin (US-Avastin) notwithstanding minor differences in clinically inactive components. The manufacture of Alympsys is well-controlled and leads to a product that is pure and potent. The conditions used in manufacturing have been sufficiently validated. It is recommended that this product be approved for human use under conditions specified in the package insert.

B. Approval Action Letter Language:

- Manufacturing location:
  - Drug Substance:
    (b) (4)
  - Drug Product:
    (b) (4)
- Fill size and dosage form:
  - 100 mg/4 mL single-dose vial, Injection, for intravenous use
  - 400 mg/16 mL single-dose vial, Injection, for intravenous use
- Dating period:
  - Drug Product:
    - 100 mg vials- 30 months at 2-8 °C
    - 400 mg vials- 30 months at 2-8 °C

- Drug Substance: (b) (4) months at (b) (4) °C

- Exempt from lot release:
  - Yes, ALYMPSYS is a specified product and exempt from lot release in accordance with 21 CFR 601.2a.

C. Assessment Summary:

ALYMPSYS (bevacizumab-maly, MB02) is a proposed biosimilar to US-licensed Avastin (US-Avastin) that is intended to be delivered by the same route of intravenous administration for the same indications as the US-Avastin reference product, other than those indications protected under the orphan exclusivity.

The data submitted support the demonstration that ALYPMSYS is highly similar to US-Avastin, notwithstanding minor differences in clinically inactive components and that the analytical portion of the scientific bridge to justify the use of clinical data derived from European Union (EU)-approved Avastin was established. (Refer to Section II of this memo for further details and discussion of the differences observed).

The assessment of manufacturing information provided in this application has concluded that the methodologies and processes used for drug substance (DS) and drug product (DP) manufacturing, release, and stability testing are sufficiently robust and controlled to ensure the consistent manufacture of a safe, pure, and potent product.

The microbial control and sterility assurance strategy is sufficient to support consistent manufacture of a sterile product and the OPMA assessors are recommending approval for this BLA from a sterility assurance and microbiology product quality perspective.

The pre-license inspection of the (b) (4) used to manufacture and test MB02 drug substance recommended approval.

Individual assessments for each discipline are located in separate documents in Panorama.

D. Recommendation on Phase 4 (Post-Marketing) Commitments, Requirements, Agreements, and/or Risk Management Steps, if approvable:
None

II. Evaluation of the Comparative Analytical Assessment and Evaluation of the Analytical Component of the Scientific Bridge

A. Analytical Assessment Overview and Conclusions

To support a demonstration that MB02 is highly similar to US-Avastin, Amneal Pharmaceuticals performed a comparative analytical assessment using 19 US-Avastin lots (10 lots of 100 mg/4 mL; 9 lots of 400 mg/16 mL), 17 EU-approved Avastin lots (8 lots of 100 mg/4 mL; 9 lots of 400 mg/16 mL) with expiration dates of US-Avastin lots ranging

from October 2017 through September 2021 and 15 independent lots of MB02 DP with ages ranging from 0 to 25 months consisting of 8 lots of 100 mg/4 mL and 7 lots of 400 mg/16 mL vials. The ages at the time of testing of US- and EU-Avastin were adequate to capture potential reference product analytical differences over time.

There were two process iterations during the commercial development stage of MB02: the SP (used in comparative clinical studies) and DM processes. These process iterations had the goal of enhancing process consistency and further improvement of the analytical similarity between MB02 and US-Avastin. All MB02-SP lots used in comparative clinical studies were included in the comparative analytical assessment. The MB02 lots used in the comparative analytical assessment all represent independent DS lots; their derivation is as follows:

- 9 lots of MB02-SP DP manufactured at commercial scale (b) (4)
- 6 lots of MB02-DM DP manufactured at commercial scale

The comparability of the lots manufactured by MB02-SP and MB02-DM processes was discussed in a BPD Type 2 meeting held with the Agency on October 14, 2020 and was assessed and found to be established based on data that was also submitted to this BLA. Small improvements were made in the MB02-SP proces (b) (4) Although the data from the proposed commercial (MB02-DM) process better aligns with US-Avastin, which strengthens the support for a demonstration that MB02 is highly similar to US-Avastin, these changes would not preclude the ability to leverage the clinical studies that were performed with MB02-SP lots as they were found to be comparable with lots manufactured by the proposed commercial process (MB02-DM).

The comparative analytical assessment was comprised of extensive comparative physicochemical and functional assessment of the quality attributes of MB02 and US-Avastin and included a comparative assessment of their degradation profiles under several relevant forced degradation conditions including thermal stress (45°C for 14 days), oxidative stress (~0.1% H_2O_2 at 2-8°C for 14 days), agitation (600 rpm for 18 hours), pH extremes (pH 3.0 for 10 days, pH 9.0 for 7 days at 2-8°C), and photo (light) stress (1.25 million lux-hr visible light, with increasing amounts of UV, at 22-28°C, 60% relative humidity).

Amneal assessed quality attributes using an approach based on risk and criticality for statistical evaluation of comparative analytical results. The highest-ranking attributes that were tested using quantitative assays were evaluated using equivalence testing. Attributes that were considered moderate for criticality that were tested using quantitative assays were evaluated using quality ranges defined by US-Avastin or EU-Avastin; these quality ranges were established by multiplying the standard deviation of the US-Avastin or EU-Avastin data by a multiplier of 2 or 3, depending on analytical method variability, criticality of the quality attributes and manufacturing variability. The standard deviation multiplier used to establish each quality range was scientifically justified. The least critical attributes, and those attributes that were tested using qualitative assays, were evaluated using a comparison of visual displays of the data. Results from method validation or qualification

studies support the suitability of the methods used in the comparative analytical assessment.

Amneal is seeking licensure of two strengths of MB02, 100 mg/4 mL and 400 mg/16 mL per vial. US-Avastin is available in two strengths, 100 mg/4 mL and 400 mg/16 mL, single-dose vials for intravenous infusion. Our assessment of the MB02 and US-Avastin data supports that MB02 has been demonstrated to be highly similar to US-Avastin, notwithstanding minor differences in clinically inactive components. MB02 has the same strength, dosage form, and route of administration as US-Avastin. Amneal used a comprehensive selection of analytical methods that were suitable to evaluate the critical quality attributes of MB02 and US-Avastin to support the demonstration that the products are highly similar. Numbers of lots tested and statistical analyses were appropriate to allow for a meaningful evaluation of the results of the comparative analytical studies. While some differences were observed in a subset of quality attributes, these differences were determined not to preclude a demonstration that MB02 and US-Avastin are highly similar.

Based on our assessment of the data, we also conclude that Amneal established the analytical component of the scientific bridge between MB02, US-Avastin, and EU-Avastin, using the same methods and statistical approaches used to evaluate similarity between MB02 and US-Avastin. The analytical component of the scientific bridge was established to justify the relevance of the data generated from studies using EU-Avastin as a comparator to the assessment of biosimilarity. Although differences were observed in certain attributes for comparisons between MB02, US-Avastin, and EU-Avastin (see section D below), the applicant provided adequate data and information to resolve the residual uncertainty raised by these differences. The observed differences do not preclude a demonstration that MB02 is highly similar to US-Avastin or the establishment of the analytical component of the scientific bridge (refer to Section IV below).

B. Results of the Comparative Analytical Assessment

Table A. Quality Attributes Analyzed in the Comparative Analytical Assessment

Physico-chemical/Functional Characteristics	Quality Attribute Assessed	Supports a Demonstration of Highly Similar	Supports the Analytical Component of the Scientific Bridge
Primary Structure	Amino acid sequence	Yes	Yes
	Intact, reduced, and reduced and deglycosylated molecular mass (RPLC-UV/MS)	Yes	Yes
	Reduced tryptic peptide mapping (LC-MS/MS)	Yes	Yes
	Sequence Variants Analysis (LC-MS)		
N-linked Glycans	High Mannose	Yes	Yes
	Afucosylation	Yes	Yes
	Terminal Galactosylation	Yes	Yes
	Sialylation	Yes	Yes

Physico-chemical/Functional Characteristics	Quality Attribute Assessed	Supports a Demonstration of Highly Similar	Supports the Analytical Component of the Scientific Bridge
Amino Acid Modifications	Methionine Oxidation (reduced tryptic peptide mapping)	Yes	Yes
	Deamidation (reduced tryptic peptide mapping)	Yes	Yes
	N-terminal Variants (reduced tryptic peptide mapping)	Yes	Yes
	C-terminal Variants (reduced tryptic peptide mapping)	Yes	Yes
	O-linked glycosylation (peptide mapping)	Yes	Yes
	Glycation (RP-HPLC-UV-MS)	Yes	Yes
Higher Order Structure	Free Thiol's (Ellman's Reagent)	Yes	Yes
	Disulfide Bridges (non-reduced peptide mapping)	Yes	Yes
	Secondary Structure (CD, data and spectral profile)	Yes	Yes
	Tertiary Structure (FTIR)	Yes	Yes
	Higher Order Structure, epitope mapping (HDX-MS)	Yes	Yes
	Structural stability (μDSC)	Yes	Yes
	Colloidal stability (temperature ramped DLS)	Yes	Yes
Product-related variants and impurities	HMW (SE-UPLC)	Yes	Yes
	Monomer (SE-UPLC)	Yes	Yes
	Purity (HC + LC) (rCE-SDS)	Yes	Yes
	NGHC (rCE-SDS)	Yes	Yes
	Purity (nrCE-SDS)	Yes	Yes
	HHL (nrCE-SDS)	Yes	Yes
	Main Peak (CEX-HPLC)	Yes	Yes
	Acidic Species (CEX-HPLC)	Yes	Yes
	Basic Species (CEX-HPLC)	Yes	Yes
Bioactivity- Fab mediated	Potency by proliferation inhibition in HUVEC	Yes	Yes
	VEGF-A165 binding by ELISA	Yes	Yes
	VEGF-A isoforms 121, 189, and 206 binding by ELISA	Yes	Yes
	VEGFA family specificity Binding to VEGFC and VEGFD by ELISA	Yes	Yes
	VEGF165 neutralization (reporter gene assay)	Yes	Yes
	VEGF165 neutralization (receptor dimerization assay)	Yes	Yes
Bioactivity- Fc mediated	FcRn binding by ELISA	Yes	Yes
	FcγRIIIa (158V) %KD by AlphaLISA	Yes	Yes
	FcγRIIIa (158F) by AlphaLISA	Yes	Yes
	FcγRIa binding by SPR	Yes	Yes

Physico-chemical/Functional Characteristics	Quality Attribute Assessed	Supports a Demonstration of Highly Similar	Supports the Analytical Component of the Scientific Bridge
	FcγRIIa (131H and 131R) binding by SPR	Yes	Yes
	FcγRIIb binding by SPR	Yes	Yes
	FcγRIIIb binding	Yes	Yes
	Macrophage Mannose Receptor (BLI)	Yes	Yes
	C1q binding	Yes	Yes
	Antibody-dependent cellular cytotoxicity (ADCC)	Yes	Yes
	Complement-dependent cytotoxicity (CDC)	Yes	Yes
		Yes	Yes
Drug Product Attributes	Protein concentration	Yes	Yes
	Deliverable volume	Yes	Yes
Comparative Forced Degradation	Temperature (45 C; 3, 7, 14 days)	Yes	Yes
	Agitation (600 rpm, 2, 6, 18 hours)	Yes	Yes
	pH (pH 3.0 and 9.0, 2-8 C, 1, 3, 7, 10 days)	Yes	Yes
	Photo stress (1.25 million lux hrs (400-800 nm) and increasing amts of UV (320-400 nm) at 22-28 C, 60% RH	Yes	Yes

C. Comparative Analytical Studies to Support the Use of a Non-US-Licensed Comparator Product

The comparative clinical study, MB02-C-02-17, supporting this application used a non-US-licensed comparator product, EU-approved Avastin. To support the relevance of these comparative clinical data to the assessment of biosimilarity, the applicant performed a three-way pairwise comparative analytical study (refer to Table A above) as well as a pharmacokinetic (PK) similarity study (MB02-A-05-18) to establish an adequate scientific bridge. The analytical component of the scientific bridge included three-way, pairwise comparison of MB02 to US-Avastin, MB02 to EU-approved Avastin, and EU-approved Avastin to US-licensed Avastin. The same analytical methods used for the assessment of similarity between MB02 and US-Avastin were used for comparison of MB02 and EU-Avastin and US-Avastin and EU-Avastin. The differences observed in the analytical studies do not preclude a demonstration that the analytical component of the scientific bridge is acceptable. See section D below. The results of the assessment establish a robust analytical component of the three-way scientific bridge.

D. Assessment of Analytical Study Results

Comparative analytical acceptance criteria for the pairwise three-way comparison between MB02, US-Avastin, and EU-Avastin were met for all attributes evaluated with the following exceptions:

- Sequence Variants: Cysteine to tyrosine substitutions (b) (4) [redacted] Because the levels of these substitutions in the commercial material are acceptably low, and because they have been demonstrated not to have a discernible impact on potency, these small differences do not preclude a demonstration that MB02 is highly similar to US-Avastin or the establishment of the analytical component of the scientific bridge.

- Free Thiol: Levels of free thiols as determined by Ellman's assay in MB02 were found to be slightly higher than those in US-Avastin and EU-Avastin. 9 out of 15 MB02 lots (Min – Max range: 0.008791 – 0.010541 mol/mol) fell outside of the US-Avastin quality range (average 0.006808 – 0.009486 mol/mol) and 10 MB02 lots were outside of the EU-Avastin range for free thiol (0.006522 – 0.009434 mol Cys/mol). These were likely at least in part due to the cysteine to tyrosine substitutions discussed above, but the free thiol levels in all batches analyzed are extremely low and the difference between MB02 and US-Avastin and EU-Avastin had no discernible impact on the disulfide bridge evaluation by non-reduced tryptic peptide mapping or secondary or tertiary structure data by multiple assays. Therefore, the differences observed in the free thiol levels of MB02 lots do not preclude a demonstration that MB02 is highly similar to US-Avastin or a determination that the analytical component of the scientific bridge was established.

- HHL Impurity by nrCE-SDS: (b) (4) [redacted] MB02 manufactured by the current process is highly similar to US-Avastin or the establishment of the analytical component of the scientific bridge.

- Protein Concentration: While 5 out of 15 lots of MB02 were higher than the QR for protein concentration of US-Avastin (25.4-27.5 mg/mL, compared to a QR of 23.2-25.4 mg/mL), and four lots fell outside of the quality range based on EU-Avastin (22.8-25.9 mg/mL), these lots were produced by the earlier MB02-SP process and subsequent process modifications have ensured that all subsequent lots of commercial scale MB02 are within an acceptable range. The differences observed in the protein concentration of MB02 lots manufactured by the earlier process do not preclude a demonstration that MB02 is highly similar to US-Avastin or a determination that the analytical component of the scientific bridge was established.

- Acidic Variants: MB02 demonstrated a wider range in its distribution with regard to acidic variants by CEX-HPLC (18.3 – 25.4%) than US-Avastin (20.8 –23.9%), with 7 out of 15 MB02 batches falling outside of the acceptance range (US-Avastin average ± 3SD: 19.9 – 25.1%), whereas all MB02 lots fell within the acceptance range for EU-Avastin (average ± 3SD: 18.3 – 26.9%). Characterization data was provided to demonstrate the relationship between the formation of acidic variants and age of the DP lot for all three products (see Figure 53 of Section 3.2.R-Biosimilarity Assessment). MB02 lots tested represent ages of 0-25 months, whereas the US-Avastin lots are thought to represent 2-17 months and EU-Avastin from 6-22 months, and the increase in acidic species appears to roughly correlate with the age of the lot, where the MB02 batches with the highest amounts of acidic species (17A043, 17A111 and 18A018) represent the oldest lots (25, 21, and 16 months, respectively). Comparative stability studies (3.2.P.8 Stability) demonstrate similar kinetics with regard to the formation of acidic species over time between MB02, US-Avastin, and EU-Avastin, and characterization of the acidic peaks did not demonstrate any significant impact on potency (Section 3.2.S.3.2 Characterization- Impurities). Taken together, these points support that the observed differences in acidic species do not preclude a demonstration that MB02 is highly similar to US-Avastin or the establishment of the analytical component of the scientific bridge.

- Afucosylated glycans: Whereas the afucosylated glycan levels of EU-Avastin (2.95-3.90%) fell within the quality range of US-Avastin (1.72-6.08%), they were on the low side of the US-Avastin quality range, whereas levels of afucosylated glycans for MB02 lots (4.29-6.00%) were on the high side, resulting in all MB02 lots falling outside of the quality range for EU-Avastin. The majority of afucosylated glycans in all three products were found to be high-mannose species, which followed the same pattern. While afucosylated glycans can increase binding to FcγRIII and ADCC, this is not expected to be a mechanism of action for bevacizumab products due to the soluble nature of the VEGF-A target. High mannose glycans can, however, affect the PK of antibodies as this glycan enables more rapid clearance. However, an average difference of 2.9% (between MB02 and US-Avastin) or 3.1% (between MB02 and EU-Avastin) would not be expected to differentially impact the PK of MB02 as compared with US-Avastin or EU-Avastin, as this degree of difference would not significantly impact clearance of monoclonal antibodies. See, e.g., Goetze AM, et al., High-mannose glycans on the Fc region of therapeutic IgG antibodies increase serum clearance in humans. Glycobiology, 2011;21:949-59. Therefore, the observed differences in afucosylation, and specifically high mannose, do not preclude a demonstration that MB02 is highly similar to US-Avastin or the establishment of the analytical component of the scientific bridge. Additionally, we conferred with the clinical pharmacology reviewers, and the PK data are consistent with these conclusions.

- Galactosylation: The levels of galactosylation of the N-linked glycan of all 15 lots of MB02 were higher than the quality ranges of either US-Avastin or EU-Avastin, whereas EU-Avastin lots were all found to be within the quality range derived from US-Avastin lots. However, similar to the case with afucosylation, while galactosylation can have an impact on C1q binding and CDC as well as FcγRIII binding and ADCC, this is not expected to be a mechanism of action for bevacizumab products due to the soluble nature of the VEGF-A target. Therefore, the observed differences in galactosylation do not preclude a

demonstration that MB02 is highly similar to US-Avastin or the establishment of the analytical component of the scientific bridge.

- Sialic acid: Levels of sialylated glycans in MB02 lots (0.3-0.7%) are all higher than those found in US-Avastin (0.0-0.3%) or EU-Avastin (0.0-0.3%). Sialyation of the conserved Fc glycan may have the potential to impact Fc functions or PK but effector functions are not believed to play a role in the potency of bevacizumab products and there are very low amounts of this modification in MB02, US-Avastin, or EU-Avastin. Therefore, the observed differences in sialylation do not preclude a demonstration that MB02 is highly similar to US-Avastin or the establishment of the analytical component of the scientific bridge.

- Glycation: Levels of glycation of MB02 lots (average of 2.3% on the light chain (LC) and 4.0% on the heavy chain (LC)) were found to be lower on all MB02 lots than both US-Avastin and EU-Avastin (3.6%, LC and 7.7%, HC for US-Avastin and 3.6% LC and 7.9% HC, EU-Avastin), placing all glycation values for MB02 below the quality ranges of both US-Avastin and EU-Avastin, which were alike. There is no known impact of glycation on US- or EU-Avastin, and characterization data in the BLA support that US- or EU-Avastin charge variants that are enriched glycated molecules do not display reduced potency. Furthermore, levels of glycation is considered a precursor to potential degradation pathways for therapeutic proteins, with lower levels having less potential for degradation. Therefore, the differences observed in the glycation of MB02 do not preclude a demonstration that MB02 is highly similar to US-Avastin or the establishment of the analytical component of the scientific bridge.

- Main Peak by SEC: Overall, slightly higher purity by SEC was found for MB02 than US-Avastin or EU-Avastin, whereby all MB02 lots (range of 97.6-99.3%) were found to be above both US-Avastin and EU-Avastin quality ranges (96.7 – 97.5% and 96.2 –97.3%, respectively); similarly, the levels of aggregates in MB02 lots (0.7-2.2%) were lower than found in US-Avastin and EU-Avastin lots (quality ranges of 2.1 – 3.2% and 2.5-3.6% for US-Avastin and EU-Avastin, respectively). These small differences would not be expected to have a biological impact on bevacizumab and do not preclude a demonstration that MB02 is highly similar to US-Avastin or the establishment of the analytical component of the scientific bridge.

- FcγRIIIa (158F and V) binding: One third of the MB02 lots (range of 124.1 – 150.7% binding compared with reference standard) were found to be within the quality ranges of US-Avastin (127.1 – 271.1%) or EU-Avastin (146.3 – 225.0%). However, as was mentioned above, FcγRIII binding and ADCC are not expected to represent a mechanism of action for bevacizumab due to the soluble nature of the VEGF-A target. MB02 lots were found to be within the US-Avastin and EU-Avastin quality ranges for all other biological assays. Therefore, the observed differences in FcγRIII binding do not preclude a demonstration that MB02 is highly similar to US-Avastin or the establishment of the analytical component of the scientific bridge.

In summary, the totality of the comparative analytical assessment supports a demonstration that MB02 is highly similar to US-licensed Avastin and, taken with the comparison between US-Avastin and EU-Avastin, establishes the analytical component of the scientific bridge to justify the relevance of data generated with EU-approved Avastin to the assessment of biosimilarity.

E. Same Strength

MB02 has the same dosage form and route of administration as US-licensed Avastin but has a different formulation. US-licensed Avastin is available in two strengths: 100mg/4 mL and 400mg/16 mL in single-dose vials[1]. Amneal is seeking approval of MB02 for the same strengths as US-licensed Avastin. Comparative protein concentration (mg/mL) was assessed as part of the comparative analytical assessment. (b) (4) Based on the comparative analytical assessment and manufacture data, the proposed strengths of 100 mg/4 mL and 400 mg/16 mL MB02 in a vial has the same total content of drug substance in units of mass in a container and the same concentration of drug substance in units of mass per unit volume as the corresponding strengths of US-licensed Avastin. Each strength of MB02 in a vial is the same as that of US-licensed Avastin.

III. Summary of Quality Assessments:

A. CQA Identification, Risk and Lifecycle Knowledge Management

Table 2: Active Pharmaceutical Ingredient CQA Identification, Risk and Lifecycle Knowledge Management

CQA (type)	Risk	Origin	Control Strategy (b) (4)	Other
Identity	Efficacy and Safety	Intrinsic to the molecule		
Potency (VEGF-A binding and neutralization, downstream inhibition of proliferation)	Potency, Efficacy, and Safety	Intrinsic to the molecule; impacted by process and stability. Oxidation, fragmentation, aggregation		
Glycosylation- high mannose (product-related impurities)	PK/Efficacy	Cell culture; affected by bioreactor conditions.		
High molecular weight (HMW) species /Aggregates (product-related impurities)	Efficacy, PK and immunogenicity	Manufacturing process and storage conditions. Minimal increase is expected on DS stability under		Aggregates are increased upon exposure to light, heat, and high pH stress.

[1] US Prescribing Information, US Licensed-Avastin. Accessed June 6, 2021 from/ https://www.accessdata.fda.gov/drugsatfda_docs/label/2020/125085s332lbl.pdf

		controlled conditions.	(b) (4)	
Low molecular weight (LMW) species (product-related impurities)	Efficacy and safety	Can be introduced during manufacture and storage		Increased upon exposure to heat, high pH stress, and light stress. (b) (4)
Oxidation	Efficacy (can impact VEGF binding), PK (can impact FcRN binding)	Manufacturing process and during storage		
FcRn binding	Efficacy, PK	Manufacturing process		
Charge Variant Profile	Efficacy, PK	Manufacturing process and during storage		

B. Drug Substance bevacizumab-maly Quality Summary

CQA Identification, Risk, and Lifecycle Knowledge Management

Table 3: Drug Substance CQA Process Risk Identification and Lifecycle Knowledge Management

CQA (type)	Risk	Origin	Control Strategy (b) (4)	Other
Bioburden	Safety, purity, and efficacy (degradation or modification of the product by microbial contamination)	Raw materials and manufacturing process		
Bacterial endotoxins (Contaminant)	Safety, purity, immunogenicity	Raw materials and manufacturing process		
Mycoplasma	Safety	Cell culture		
Viral contamination	Safety	Raw materials, cell culture		

			(b) (4)
Protein concentration	Efficacy	DP manufacturing	
Host cell DNA	Safety and immunogenicity	Cell culture	
Host cell protein	Safety and immunogenicity	Cell culture	
(b) (4)	Safety and immunogenicity	(b) (4)	
	Safety		
	Safety and immunogenicity		

- **Description:**
  MB02 is a recombinant humanized IgG1 kappa monoclonal antibody produced in CHO cells consisting of two heavy chains (453 amino acids each) and two light chains (214 amino acids each). The antibody targets human vascular endothelial growth factor A (VEGFA). The bevacizumab-maly DNA sequence was derived from literature and the sequence information was used as a template to synthesize the corresponding DNA sequences for the HC and LC variable regions. The DNA sequence derived from literature was verified by peptide mapping using US-licensed Avastin. MB02 has an average molecular weight of 149 kDa. It has the typical structure of an IgG1 antibody with 4 interchain disulfide bonds and 12 intrachain disulfide bonds and typical N-linked glycan structures in the heavy chain at Asn 303 that are predominantly core fucosylated, complex-type, bi-antennary structures, G0F/G0F (major species), and G0F/G1F, G1F/G1F, G1F/G2F, or G0F/G2F.

- **Mechanism of Action (MoA):**
  MB2 binds specifically to VEGF-A and prevents the interaction of VEGF-A with its receptors, thereby inhibiting the known functional activities of VEGF-A. According to the Avastin USPI, preventing the normal biological activity of VEGF-A regresses existing vascularization of tumors, inhibits formation of new tumor vasculature and normalizing remaining tumor vasculature, thereby inhibiting tumor growth. The same MoA of bevacizumab, i.e. inhibition of VEGF-A-induced angiogenesis and vascular permeability, is identified for each of the approved indications.

  VEGF-A, a member of the cysteine knot growth factors family of proteins, is responsible

for regulating vasculogenesis and angiogenesis under both normal (e.g. developmental and wound repair functions) and pathophysiological (e.g. tumor growth) conditions. VEGF-A provides several functions that are important for angiogenesis and include induction of endothelial cell proliferation and survival, increase in vascular permeability, and chemotaxis and homing of bone marrow cells for hematopoiesis. The main identified receptors that bind VEGF-A and mediate vasculogenesis/ angiogenesis and chemotaxis/hematopoiesis are VEGF receptor 2 (VEGFR2/ KDR) and receptor 1 (VEGFR1/Flt-1), respectively. VEGF-A can exist in several isoforms due to differential splicing that exert local as well as distal signaling events. Most splice isoforms larger than VEGF-A148 contain a significant part of the heparin binding site sequence and thus are anticipated to have some association with heparin on the cell surface. VEGF-A splice isoforms 165 and larger isoforms also contain a binding site for the neuropilin co-receptors that enhances VEGF signaling. The VEGF-A epitope recognized by US-licensed Avastin and SB-8 is conserved in all splice isoforms tested.

- **Potency Assay:**
  Amneal Pharmaceuticals proposes to use two potency assays for MB02. The first is an ELISA-based binding assay designed to measure the relative binding activity of MB02 DS and DP to VEGF-A (the VEGF 165 isoform) as compared to an MB02 reference standard. In this assay, plates with 96 wells are blocked with a given amount of reference standard overnight. Serial dilutions of the samples and the reference standard are diluted with a fixed amount of VEGF, incubated, and added to the blocked plate. After washing the plate, the VEGF that has not been neutralized reacts with a biotinylated anti-VEGF antibody. The immobilized MB02-VEGF-biotinylated antibody complex is detected and measured using avidin-horseradish peroxidase (HRP), which forms a colored product in the presence of the TMB substrate to which sulfuric acid is added. The measurement of the absorbance at a wavelength of 450 nm quantitates the relative binding activity of bevacizumab to rhVEGF-A. In the absence of a competitor, maximum absorbance is obtained by binding to the maximum possible quantity of VEGF. As the concentration of the competitor increases, a greater quantity of VEGF is neutralized, thus reducing the VEGF available for binding to the well. The data is fit using a 4-parameter logistic model. The relative inhibitory activity, and confidence intervals of the sample are calculated using parallel line analysis.

  The potency assay used to measure MB02 activity is a cell-based anti-proliferation assay using human umbilical vein endothelial cells (HUVEC). HUVEC cells express VEGF-A receptors, VEGFR1, VEGFR2, VEGFR3, as well as NRP-1 and NRP-2, and proliferate in the presence of exogenously added VEGF-A. Serial dilution of MB02 reference standard or samples enables a dose dependent inhibition of VEGF165-induced proliferation of HUVEC cells in this assay. This inhibition is compared to an internal reference standard. The read out is based upon the fluorescence of a luciferase-based reagent that measures viability by reacting with intracellular ATP and causing a luminescent signal proportional to the number of viable cells.

- **Reference Materials:**
  Amneal Pharmaceuticals has created a two-tiered reference standard system. The primary reference standard (PRS, (b) (4)) was generated from (b) (4) by a previous process. The Second Interim Reference

Standard (IRS; (b) (4)), was used to qualify the PRS and was made by the same process. The Secondary Reference Standard (SRS, (b) (4)) was manufactured from the (b) (4) process used to manufacture material used in clinical studies.

The qualification of the RRS, IRS, and PRS were adequate. Qualification parameters for future reference standards are included in the BLA. The stability program for the PRS and SRS is adequate.

- **Critical starting materials or intermediates:**

(b) (4) Raw materials used were of non-animal origin. The WCB was adequately tested to ensure product safety from adventitious agents.

- **Manufacturing process summary:**

(b) (4)

- **Container closure:**

(b) (4)

- **Dating period and storage conditions:**

The dating period for the drug substance is (b) (4) months when stored at (b) (4) °C. Thirty-six months of real time stability results were provided for clinical stability lots manufactured by a process representative of the intended marketing process, as well as 12 months of data for PPQ lots. Accelerated and stressed stability data also support that (b) (4) month expiration dating can be given to DS stored at (b) (4) °C based on the data available. The post-approval DS stability protocol proposes to place one lot of DS annually on stability.

C. Drug Product Alymsys Quality Summary:

Table 4 provides a summary of the identification, risk, and lifecycle knowledge management for drug product CQAs that derive from the drug product manufacturing process and general drug product attributes. For additional information, see the OBP quality technical assessment and the Drug Product Microbiology and Facilities technical assessment in Panorama.

Table 4: Drug Product CQA Identification, Risk, and Lifecycle Management

CQA (type)	Risk	Origin	Control Strategy	Other
Sterility (Contaminant)	Safety (infection), purity, and efficacy via degradation or modification of products by microbial contamination	Manufacturing process or failure of container closure integrity	(b) (4)	
Endotoxin (Contaminant)	Impact on safety, purity, and immunogenicity	Contamination may be introduced throughout DP manufacturing process		
Container Closure Integrity (Contaminant)	Impact on safety (breach in the container closure system may impact product sterility)	May be impacted by shipping or storage conditions		
Extractable Volume	Efficacy	DP manufacturing		
Protein concentration	Efficacy	DS/DP manufacturing		
Particulate matter	Impact on immunogenicity and safety	DS/DP manufacturing		
Sub-visible particulates	Impact on immunogenicity and safety	DS/DP manufacturing		
pH	Impacts product stability and potentially PK/PD	Formulation, DS/DP manufacturing		

Osmolality	Stability, safety	DS/DP manufacturing	(b) (4)	
Leachables (Process-related impurity)	Safety	Manufacturing equipment and container closure system (CCS)		An additional accelerated leachables study was performed to support the safety of the CCS with MB02 (b) (4)

- **Potency and Strength:**
  Potency for MB02 is defined as the percent of relative binding of VEGF-A by ELISA compared with MB02 reference material or percent inhibition of proliferation relative to MB02 reference material. The potency assays are the same as those described in the Drug Substance section of this review.

  The strength is 25 mg/mL, and MB02 DP is supplied in two size vials for intravenous infusion: a 100 mg/4 mL sterile, preservative-free solution in a single-dose vial to deliver 4 mL, or a 400 mg/16 mL sterile, preservative-free solution in a single-dose vial to deliver 16 mL.

- **Summary of Product Design:**
  Alymsys is a sterile 25 mg/mL bevacizumab-maly liquid filled into (b) (4) glass vials. The drug product is supplied as a 100 mg or 400 mg, sterile, single-dose, preservative-free solution in a vial to deliver 4 mL or 16 mL of bevacizumab-maly for intravenous infusion. The container closure system consists of a 4 mL (100 mg) or 16 mL (400 mg) (b) (4) glass vial, (b) (4) rubber stopper, and aluminum seal with a plastic flip off cap.

- **List of Excipients:**
  There are no changes in excipients from the DS. Each vial of Alymsys contains 25 mg/mL of bevacizumab-maly, 60 mg/mL trehalose dihydrate, 5.8 mg/mL monobasic sodium phosphate, monohydrate, 1.2 mg anhydrous dibasic sodium phosphate, and 0.4 mg/mL polysorbate (b) (4) at pH 6.2. Additional excipient information is located in the Product Quality primary technical assessment in Panorama.

- **Reference Materials:**
  Reference materials for the DP are the same materials as used for the DS.

- **Manufacturing process summary:**
  (b) (4)

(b) (4)

- **Container closure:**
The 100 mg/4 mL Alymsys drug product is supplied in 4 mL (b) (4) glass vial with a 20 mm (b) (4) elastomer stopper (b) (4) a 20 mm aluminum seal and a yellow flip-off cap (b) (4) The 400 mg/16 mL Alymsys drug product presentation is suppled in a 20 mL (b) (4) glass vial with a 20 mm (b) (4) elastomer stopper (b) (4) a 20 mm aluminum seal, and a purple flip-off cap (b) (4)

- **Dating period and storage conditions:**
Amneal Pharmaceuticals proposed Drug product stability data were provided for both the 100mg and 400 mg vial presentations. The expiration dating for both presentations on approval will be 30 months when stored at 2-8 °C. The BLA contains an annual stability protocol for each presentation of the drug product.

D. Biopharmaceutics Considerations: N/A

E. Novel Approaches/Precedents: None

F. Any Special Product Quality Labeling Recommendations:
Single-dose vial. Store at 2-8°C. Protect from light.

G. Establishment Information:

Overall Recommendation:					
DRUG SUBSTANCE					
Function	**Site Information**	**DUNS/FEI Number**	**Preliminary Assessment**	**Inspectional Observations**	**Final Recommendation**
DS manufacturing, in-process and release and stability testing, storage of MCB and WCB, preparation of WCB	(b) (4)		PLI	VAI	Approved based on PLI
Harvest mycoplasma, MMV, and 28 day IVV testing			Facility adequate	N/A	Approve
Harvest mycoplasma, MMV, and 28 day IVV testing			Facility adequate	N/A	Approve
Harvest mycoplasma, MMV, and 28 day IVV testing			Facility adequate	N/A	Approve
DRUG PRODUCT					
Function	**Site Information**	**DUNS/FEI Number**	**Preliminary Assessment**	**Inspectional Observations**	**Final Recommendation**

DP manufacturing, in-process and release and stability testing, storage of DP, primary and secondary packaging	(b) (4)	Document audit inspection via 704(A) in lieu of a PLI.	N/A	Approve
DP release testing, batch release, and storage and distribution of DP		PLI	VAI	Approved based on PLI

H. Facilities:

(b) (4) is responsible for the manufacture of the MB02 drug substance. This facility has never been inspected by the FDA, but was inspected by (b) (4) inspection covered the (b) (4) Manufacturing area at (b) (4) QC lab, storage, and didn't visit (b) (4). For the manufacturing process, it mainly covered production and quality control documentation of (b) (4) and MB02 Bevacizumab validation reports. At the time of inspection, the MB02 monoclonal antibody (Bevacizumab) product quality review is not available as commercial batches of the antibody have not been manufactured. Approval is recommended for DS manufacturer (b) (4)) based on the pre-license inspection (PLI) conducted on (b) (4) with classification as VAI.

The Agency requested pre-inspection audit documents under Section 704(a)(4) in support of the DP manufacturing and testing at (b) (4) A records review was performed for this BLA and the requested documents were reviewed and no significant issues were identified (refer to the review of the manufacturing records in CMS WA 423520.

Final facility recommendation: Approve

I. Lifecycle Knowledge Management:

a. Drug Substance:

i. Protocols approved:

Protocols Included in BLA	Section	Proposed reporting category
Qualification of future WCB	3.2.S.2.3	Annual reportable
Qualification of future Reference Standard	3.2.S.5	
At-scale verification of (b) (4) purification process	3.2.S.2.5	
Protocol for (b) (4) purification process	3.2.S.2.5	

Registrational stability protocol for MB02 DS	3.2.S.7.1	

ii. **Outstanding assessment issues/residual risk:** None
iii. **Future inspection points to consider:** None

b. Drug Product

i. **Protocols approved:** Registrational stability protocol for MB02 DP
ii. **Outstanding assessment issues/residual risk:** None
iii. **Future inspection points to consider:** None

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/s/

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